

## Asia Pharma 2016 : Antifibrotic effects of gallic acid on activation/proliferation of cultured hepatic stellate cells and in thioacetamide-induced liver fibrogenesis in rats - Naglaa M El-Lakkany - Cairo University

Naglaa M El-Lakkany

Cairo University, Egypt

Liver fibrosis, a major cause of morbidity and mortality worldwide, is a wound-healing response to repeated liver injury, driven by different causes such as chronic hepatitis, autoimmune disease, alcoholic and nonalcoholic fatty liver diseases.<sup>1</sup> It results in impaired hepatic regenerative capacity that ultimately leads to cirrhosis and hepatocellular carcinoma.<sup>2</sup> Liver fibrosis is characterized by excessive deposition of extracellular matrix (ECM), culminating to major changes in liver architecture where ECM acts as a reservoir for pro-inflammatory and pro-fibrogenic mediators.<sup>3</sup> Hepatic stellate cells (HSCs) are the key fibrogenic effector cell type in the liver and the main ECM-producing cells.<sup>4</sup> When hepatic injury persists, HSCs become activated and transform into myofibroblast-like cells.<sup>5</sup> Activation of HSCs is promoted by tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), transforming growth factor- $\beta$  (TGF- $\beta$ ), and reactive oxygen species (ROS) produced by apoptotic hepatocytes and Kupffer cells, which in turn, secrete pro-inflammatory cytokines and further perpetuating an inflammatory state.

Materials and methods include The peels of pomegranates were manually removed, shade-dried and powdered to yield 1 kg, which was extracted with a Soxhlet extractor using methanol for 4 h according to the method of Singh et al.<sup>24</sup> The extract was filtered to remove the peels particles and then was concentrated under vacuum at 40 °C. The dried extract powder (300 g) was further used for isolation of GA. The extract was fractionated over a diaion HP $\alpha$ 20 AG column, then elution was carried out using water and methanol in different ratios, and the solvent in each fraction was removed by evaporation under reduced pressure and monitored by TLC. The fraction containing GA (75% methanol in water v/v) was purified over several sephadex LH $\alpha$ 20 columns using methanol or water-methanol (1:1 v/v) as eluent. Male Sprague-Dawley rats (Animal house, Theodore Bilharz Research Institute, Giza, Egypt) weighing 250–300 g were used in this experiment. The animal protocol was designed to minimize pain or discomfort to the animals. Rats were housed under an environmentally controlled room at 20–22 °C, 12 h light/dark cycle and 50–60% humidity with free access to food and water ad libitum throughout the acclimatization and experimental periods. All animals were euthanized by a lethal intraperitoneal injection of 10% chloral hydrate for blood and tissue collections. hepatocytes were freshly isolated from rats by a two-step portal collagenase perfusion of the liver as previously described.<sup>26</sup> On 96-well plates, both hepatocytes and HSCs were seeded in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, USA) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100  $\mu$ g/mL streptomycin at 37 °C under a humidified atmosphere of 5% CO<sub>2</sub> and 95% air. A stock solution of GA was dissolved in a small volume of dimethyl sulfoxide (DMSO, equivalent to < 1% of the final volume), filtered through a 0.22  $\mu$ m membrane and aliquots were

stored at 4 °C protected from light. Micro cultures of 5  $\times$  10<sup>3</sup> HSCs or hepatocytes were cultured in 96-well tissue culture plates (Nunc, Roskilde, Denmark) in 200  $\mu$ L DMEM supplemented with 10% FBS. After 24 h, cells were treated with different concentrations of GA (0–300  $\mu$ g/mL) for 24 and 48 h where cell survival ratios corresponding to untreated cells were examined. Each test was performed in triplicate. The anti-proliferative effect of GA on HSCs proliferation was assessed using sulforhodamine base (SRB) assay. HSCs activation was assessed by alpha-smooth muscle actin ( $\alpha$ -SMA) expression according to the manufacturer's instructions. Thirty-two male rats were randomly divided into four groups, 8 each. TAA was injected intraperitoneally (200 mg/kg, twice a week for 12 weeks) to groups of rats II, III, and IV, while group I was injected intraperitoneally with the same amount of saline and served as normal control. Rats in groups III and IV were administered silymarin (50 mg/kg)<sup>29</sup> and GA (50 mg/kg)<sup>30</sup> via oral gavage, respectively for 8 weeks starting from the 5th week of TAA-intoxication where an apparent stage of fibrosis (S2) was recorded via histopathological examination of hepatic tissues. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined spectrophotometrically<sup>31</sup> using the commercially available kits (Spectrum, Egypt). The level of reduced glutathione (GSH) and the extent of lipid peroxidation (MDA) were estimated in liver homogenates according to the methods described by Ellman<sup>32</sup> and Ohkawa et al.<sup>33</sup> respectively. Tissue inhibitor matrix metalloproteinases, type 1 (TIMP-1), transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) and platelet-derived growth factor-B (PDGF-B) levels were measured in liver tissue homogenates by commercial ELISA kit (R&D system, MN, USA). The HP content was determined in liver tissue samples as described by Woessner.<sup>34</sup> Briefly, liver tissues (300 mg) were hydrolyzed in 6 N HCl for 18 h at 110 °C and aliquots were dried and incubated with 50% isopropanol and chloramine-T solution followed by reaction with p-methylaminobenzaldehyde (Ehrlich's solution) at 60 °C for 20 min. The Results are Spectroscopic data and identification of GA, Effect of GA on HSCs proliferation, morphology and hepatocytes viability, Effect of GA on liver functions and hepatic oxidative stress, Effect of GA on liver fibrosis markers and HP content, Effect of GA on liver histology, Effect of GA on blockade of HSCs activation and induction of apoptosis, Effect of GA on proliferation of hepatocytes, HSCs play an important role in orchestrating hepatic fibrosis progression making them an appealing target for antifibrotic therapy, which upon injury are activated and acquire a myofibroblast-like phenotype accompanied by increased proliferation and ECM synthesis.<sup>39</sup> Suppression of HSC activation, proliferation and induction of their apoptosis has been proposed as therapeutic targets against hepatic fibrosis. Accordingly, this study focuses on the antifibrotic effects of GA,

clarifying its possible mechanisms on HSCs through in vitro and in vivo investigations.

Biography :

Naglaa M El-Lakkany has completed her PhD from Ain Shams University, Faculty of Science. She is the Head of Pharmacology Department, TBRI. She shared in establishment of "Drug Evaluation and Discovery Unit" and is one of the Senior Staff of the "ANDI Centre of Excellence on anti-trematodal R&D". She has published 25 articles in peer review journals. She awarded the TBRI best research articles

2011, 2012 and the TBRI Excellence award 2014. She was included in Marquis Who's Who in Medicine and Health care, 2009-2010, in the International Health Professional of The Year 2010 and Selected for the institute's WOMAN OF THE YEAR 2011. She awarded in 2015 an appreciation certificate as a recognized reviewer in all Elsevier Journals.

n.ellakkany@tbri.gov.eg